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Identification of Melatonin in Different Organs of the Cricket, *Gryllus bimaculatus*

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ABSTRACT—The possible presence of melatonin (N-acetyl-5-methoxytryptamine) was investigated in different tissues and organs of adult crickets by the use of reversed-phase high-performance liquid chromatography with fluorometric detection and radioimmunoassay. Melatonin was detected in the compound eye, antenna, ovipositor, palp, cercus, leg, wing, brain, ovary, digestive tube and Malpighian tube of the crickets, indicating that melatonin was very widely distributed in the crickets. The results suggest that melatonin in these organs might be involved in the local control of various aspects of rhythmic activity.

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) was originally found in the vertebrate pineal gland, where its synthesis and secretion exhibit a diurnal rhythm with peak activity at night [1]. It is now widely accepted that the melatonin rhythm of the pineal gland mediates photoperiodic information [18]. Melatonin has also been found in extrapineal organs of vertebrates, such as the retina [9, 19, 24], the Harderian gland [19, 24], the gastrointestinal tract [5] and the brain [13, 17, 20]. However, the function of melatonin in these extrapineal organs remains to be elucidated.

Recently, the presence of melatonin has been demonstrated in some invertebrate species including insects [22]. In insects, melatonin has been identified in the compound eye of the locust [23] and the head of the fruit fly [8]. However, the presence of melatonin in organs other than the compound eye or the head of insects has not yet been reported. The present study investigated the possible presence of melatonin in different tissues and organs of adult crickets, *Gryllus bimaculatus*, using reversed-phase high performance liquid chromatography (RP-HPLC) with fluorometric detection and radioimmunoassay (RIA).

MATERIALS AND METHODS

Animals and preparation of tissues and organs

Adult crickets were maintained under a 12 h light-12 h dark cycle (lights on at 06:00 a.m.) and ambient temperature of 24°C, with food and water *ad libitum* for a minimum of 1 week. The compound eyes, antennae, ovipositors, palpi, cerci, fore-, mid- and hind-legs, wings, brains, ovaries, digestive tubes, Malpighian tubes, and fat bodies were dissected from 20 adult females between 09 : 00 a.m. and 12 : 00 noon. The tissues and organs were weighed and homogenized in 2.5 times their volume of ice-cold 0.1 M perchloric acid containing 0.1% ascorbic acid. After centrifugation at 12000 g for 15 min at 4°C, the supernatant was extracted with 4 volumes of chloroform. The chloroform phase was washed twice with 1 ml of distilled water, evaporated and stored at −80°C until RP-HPLC analysis.

RP-HPLC analysis

The tissue or organ extracts were dissolved in 50 μl of 30% methanol in a 50 mM ammonium acetate solution (pH 4.2) for the mobile phase of RP-HPLC. Aliquots of 20 μl of the solution were directly injected into the RP-HPLC system, which was equipped with a Superiox ODS S-5 μm column (4.6×150 mm) (Shiseido Co., Tokyo) and a RF-550 fluorometric detector (Shimadzu Co., Kyoto). The detector was operated at an excitation wavelength of 280 nm and an emission wavelength of 340 nm. The flow rate of the mobile phase was adjusted to 1 ml/min and one-min fractions were collected. All separations were performed isocratically at 24°C. Melatonin and other indole standards were obtained from Sigma Chemical Co. (St. Louis, MO).

RIA of melatonin

Aliquots of RP-HPLC fractions were evaporated and dissolved in 250 μl of 10 mM phosphate buffered saline (pH 7.4) containing 1% bovine serum albumin. The melatonin content of each fraction was determined by RIA [11]. N-[2-aminoethyl-2-^{3}H] melatonin was obtained from Dupont/New England Nuclear (Wilmington, DE). Anti-melatonin serum was provided by Prof. K. Wakabayashi, Gunma University.

RESULTS

Figure 1 shows a typical elution profile of various indole standard solutions; a peak of the melatonin standard appeared at 15.7 min and was clearly separated from those of the other indole compounds examined. Figures 2–7 show elution and RIA profiles obtained from the various organ extracts of the crickets by RP-HPLC and RIA. A peak of melatonin in the elution profiles was tentatively identified by comparison of the retention time with that of the melatonin

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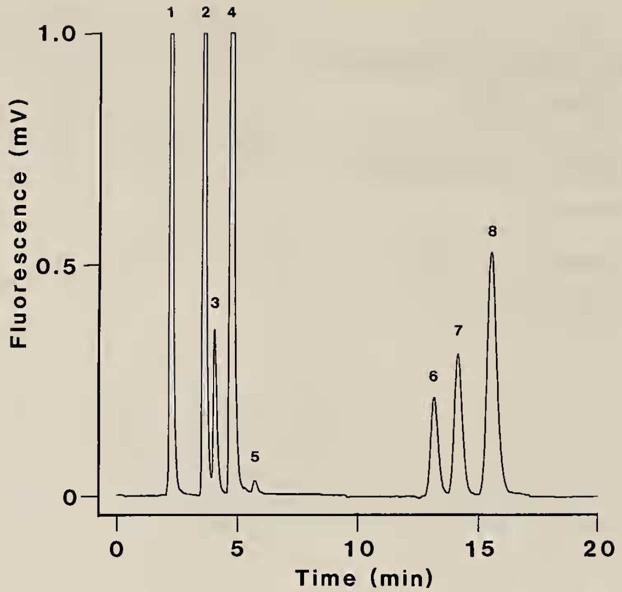

Fig. 1. RP-HPLC elution profile of various indole standards (250 pg). For chromatographic conditions, see text. Peaks: 1) 5-hydroxytryptamine (serotonin); 2) 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophol; 3) N-acetylserotonin; 4) 5-methoxytryptamine; 5) 6-hydroxymelatonin; 6) 5-methoxyindole acetic acid; 7) 5-methoxytryptophol; 8) N-acetyl-5-methoxytryptamine (melatonin).

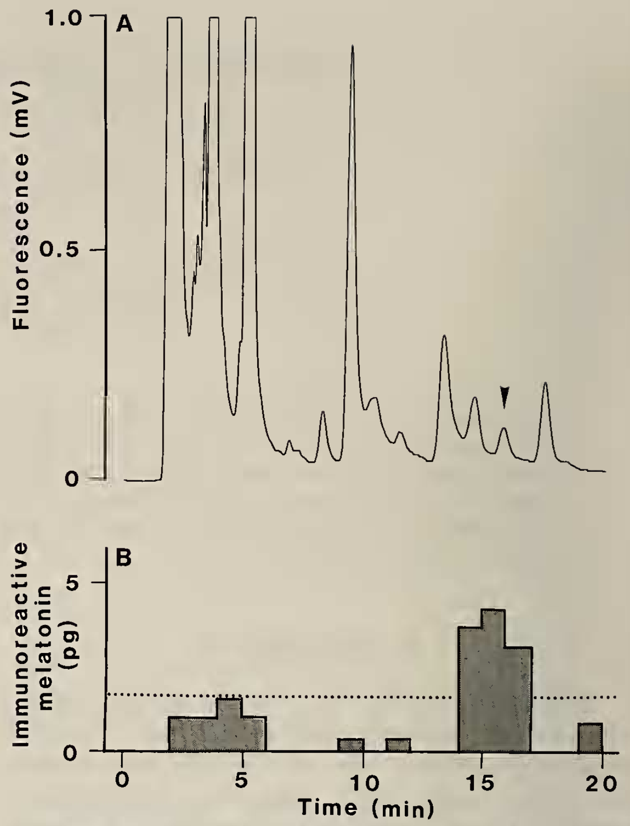

Fig. 3. RP-HPLC elution profile (A) and RIA profile (B) of the antenna extracts of the crickets.

standard as shown in Figure 1. A peak with the identical retention time to that of the melatonin standard was found in extracts of the compound eyes, antennae, ovipositors, brains and ovaries indicated in elution profiles of Figures 2–6. However, no peak with an identical retention time to that of the melatonin standard was detected in the fat body (Fig. 7A). Peaks with identical retention times to those of the other indole standards were also found in the elution profiles obtained from the extracts of these organs.

RIA revealed that the major immunoreactive peak at about 15.7 min corresponded to the endogenous melatonin detected by RP-HPLC, although the peak was broad in some extracts and minor peaks sometimes appeared in the neighborhood of the major peak (Figs. 2–6). The retention time obtained by RP-HPLC and the immunoreactivity detected by RIA confirmed that the peak observed at 15.7 min was due to melatonin. Thus, it was demonstrated that melatonin is present in the compound eye, antenna, ovipositor, brain and ovary as shown in Figures 2–6, and in addition, in the palp, cercus, legs, wing, digestive tube and Malpighian tube, although the elution profiles obtained are not shown. Table 1 shows the melatonin content (pg/cricket) and concentration

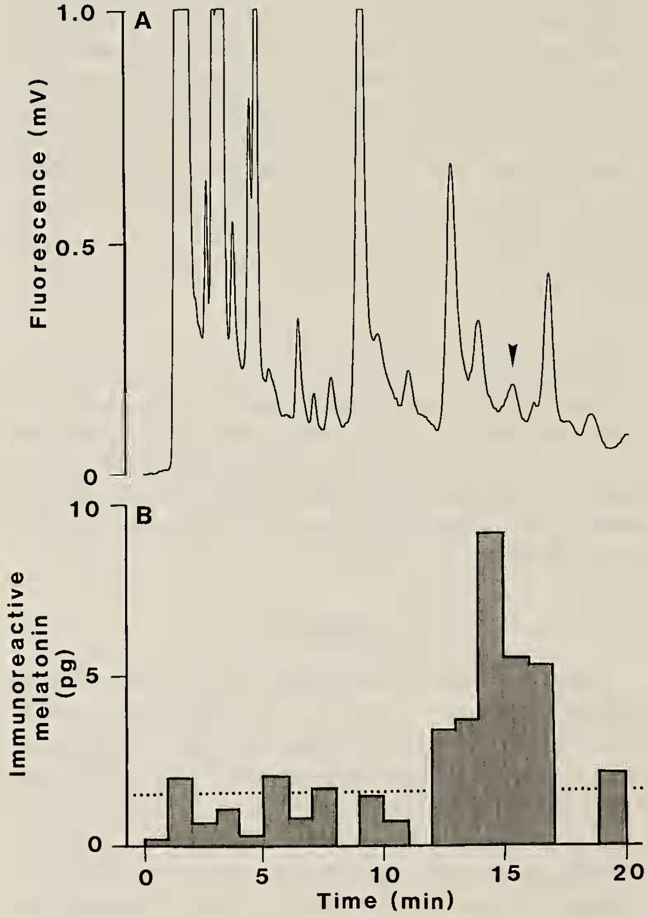

Fig. 2. RP-HPLC elution profile (A) and RIA profile (B) of the compound eye extracts of the crickets. Arrow head in (A) of Figs. 2–7 indicates the elution position corresponding to the melatonin standard. The dotted line in (B) of Figs. 2–7 indicates the detection limit of RIA (1.60 pg/100 μl).

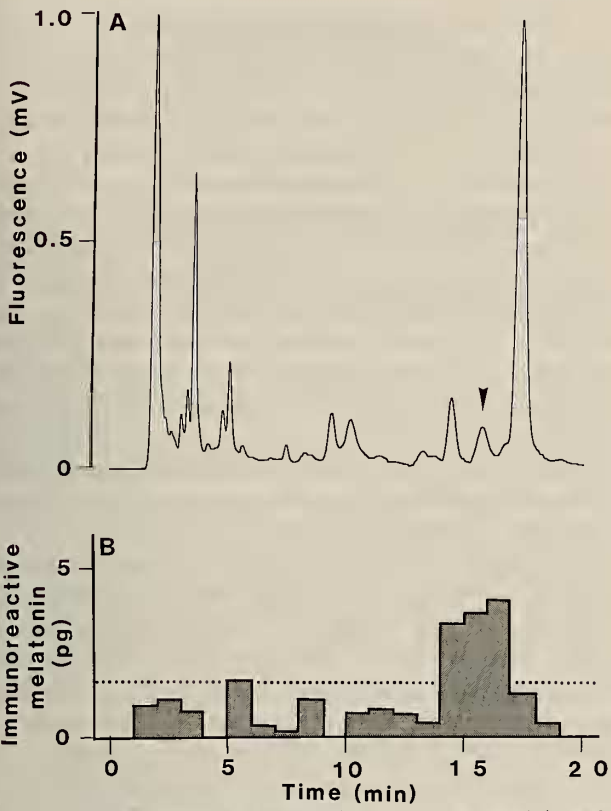

FIG. 4. RP-HPLC elution profile (A) and RIA profile (B) of the ovipositor extracts of the crickets.

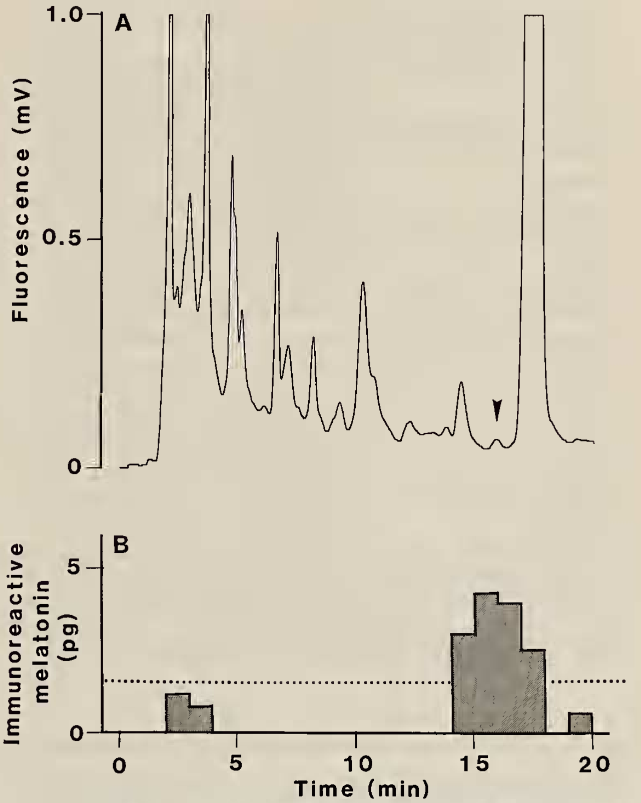

FIG. 6. RP-HPLC elution profile (A) and RIA profile (B) of the ovary extracts of the crickets.

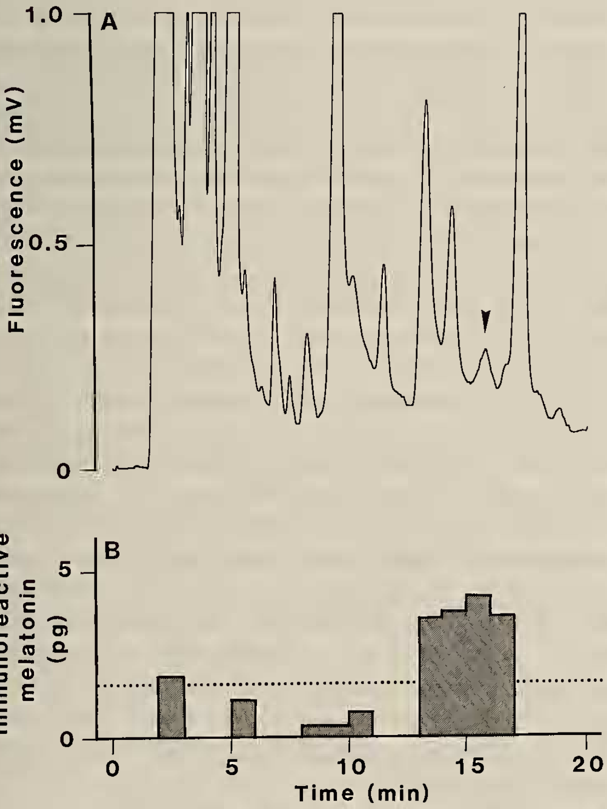

FIG. 5. RP-HPLC elution profile (A) and RIA profile (B) of the brain extracts of the crickets.

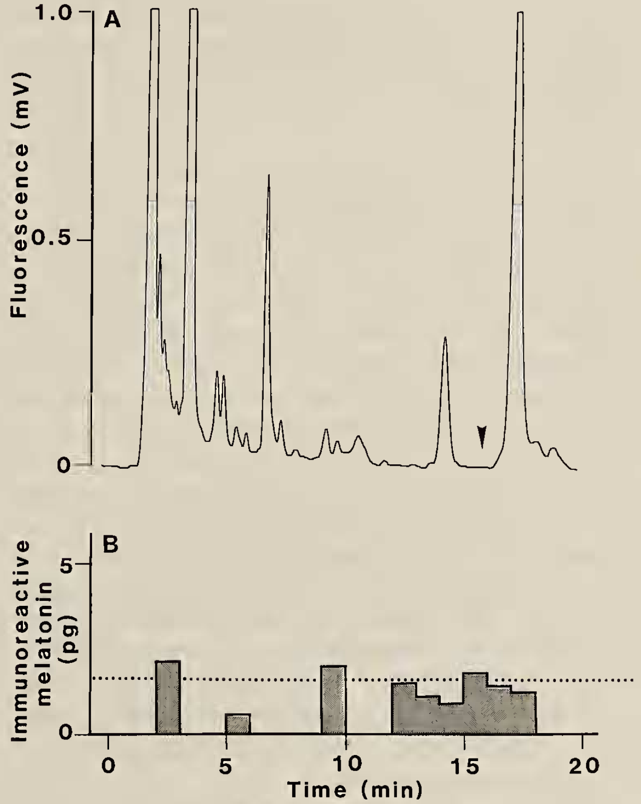

FIG. 7. RP-HPLC elution profile (A) and RIA profile (B) of the fat body extracts of the crickets.

Table 1. Melatonin Content and Concentration in Different Organs of the Adult Cricket

Organ	Content (pg/cricket)	Concentration (pg/g wet tissue)
Compound eye	5.43±1.15	263.48±49.25
Antenna	2.20±0.25	457.50±84.30
Ovipositor	1.91±0.18	240.18±38.81
Palp	2.66±0.50	122.27±16.54
Cercus	2.03±0.32	189.44±27.78
Fore-leg	1.88±0.10	117.32±24.25
Mid-leg	2.15±0.21	104.29± 7.19
Hind-leg	2.51±0.43	37.47±11.42
Wing	1.14±0.19	104.98± 9.78
Brain	1.61±0.10	83.12± 7.97
Ovary	1.91±0.09	30.08± 2.14
Digestive tube	1.70±0.17	20.75± 4.48
Malpighian tube	2.21±0.06	154.68±14.48

Each melatonin content and concentration were calculated by collecting the immunoreactivities of fraction No. 15–17 in the elution profiles obtained. Values are expressed as means and SEM derived from three separate experiments.

(pg/g wet tissue) calculated from the RIA profiles obtained from various organs of the crickets. The concentration in the sense organs, such as compound eye, antenna, ovipositor, palp, cercus, fore- and mid-legs, were usually higher than those of ovary and digestive tube.

Immunoreactive melatonin was unexpectedly detected in the fat body (Fig. 7B), although no peak of melatonin was observed in the elution profile obtained from the fat body (Fig. 7A), and its level was close to the RIA detection limit (1.60 pg/100 μl).

DISCUSSION

The present study gives clear proof of the presence of melatonin in different organs of adult crickets (Figs. 2–6 and Table 1). The proof of the presence of melatonin was obtained by two different methods, RP-HPLC with fluorometric detection and RIA. Since fluorometric detection is quite selective and highly sensitive for indole compounds [14], RP-HPLC with fluorometric detection is well suited to analysis of melatonin or other indole compounds in tissue and organ extracts of insects. However, the major immunoreactive peak at about 15.7 min was broad in some extracts, and weak immunoreactivities were observed at positions which differed from that of the melatonin standard (Figs. 2–6). This indicated that some chemical compounds or indoles other than melatonin in the crickets might interfere with its detection by RIA. Therefore, the melatonin content and concentration in the crickets was determined by collecting only those fractions corresponding to the melatonin peak, excluding all other fractions.

Melatonin in insects has previously been identified in the compound eye of the locust [23] and the head of the fruit fly [8]. The results obtained in the present study were consistent with these previous reports and revealed the presence of melatonin in other organs of the crickets. Melatonin in these organs is considered to be due to endogenous synthesis or uptake from a circulating pool. Activity of N-acetyltransferase, a key enzyme controlling melatonin synthesis in vertebrates, has been found in the brain of the fruit fly [6]. This suggests that melatonin may be synthesized in the brain of crickets. However, N-acetyltransferase activity in organs other than the brain is still unknown in insects. The peaks at about 2.3 and 4.3 min in the elution profile, as shown in Figures 2–7, corresponded to serotonin and N-acetylserotonin, respectively, the precursors of melatonin in vertebrates. This suggests that these precursors are also probably present in the melatonin-containing organs of the cricket and that melatonin may be synthesized in these organs via the same pathway as in vertebrates.

Several reports of the detection of melatonin in different organs of vertebrates have been published, as described in the Introduction. The functions of melatonin in extrapineal organs remain unknown. Several papers suggest that the extrapineal organs of vertebrates also exhibit diurnal rhythms associated with melatonin [17, 21, 25] or N-acetyltransferase activity [3, 4, 9]. Retinal melatonin has been supposed to play a role in circadian regulation of retinal physiology, such as pigment aggregation in the pigment epithelium [16] and shedding of outer segment discs [2]. In addition, gastrointestinal melatonin has been considered to inhibit gastrointestinal motility [10] and sodium absorption in the colon [15]. Based on these studies on vertebrates, we suggest that the melatonin-containing organs of the cricket may rhythmically synthesize melatonin, which may be involved in the local control of various aspects of rhythmic activity. It is also likely that melatonin in these organs is released into the blood and contributes to maintaining the melatonin levels in the systemic circulation, as has been proposed by Huether [12].

Melatonin was detected in various sense organs of the crickets, such as the compound eye, antenna, ovipositor, palp, cercus, fore- and mid-legs (Figs. 2–4 and Table 1) and, moreover, the melatonin concentrations in these sense organs were higher than those in ovary and digestive tube, as shown in Table 1. Sense organs are known to react directly to light, temperature, sound, touch, airmovement, chemical substances, etc. Melatonin inhibits the release of dopamine in rabbit retina [7] and rat hypothalamus [26]. Therefore, melatonin may modulate the activity of sensory cells and the transmission of signals from sensory cells to the central nervous system by influencing the release of neurotransmitters. Melatonin was also detected in the brain of the crickets (Fig. 5). From these facts, we might speculate that melatonin is implicated in the reception, transduction, transmission and integration of environmental information. The significance of the presence of melatonin in different organs should be further investigated.

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REFERENCES

1 Axelrod J (1974) The pineal gland: a neurochemical transducer. Science 184: 1341–1348

2 Besharse JC, Dunis DA (1983) Methoxyindoles and photoreceptor metabolism: Activation of rod shedding. Science 219: 1341–1343

3 Besharse JC, Iuvone PM (1983) Circadian clock in *Xenopus* eye controlling retinal serotonin N-acetyltransferase. Nature 305: 133–135

4 Binkley S, Hryshchyshyn M, Reilly K (1979) N-acetyltransferase activity responds to environmental lighting in the eye as well as in the pineal gland. Nature 281: 479–481

5 Bubenik GA (1980) Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm Res 12: 313–323

6 Dewhurst SA, Croker SG, Ikeda K, McCaman RE (1972) Metabolism of biogenic amines in *Drosophila* nervous tissue. Comp Biochem Physiol 43: 975–981

7 Dubocovich ML (1983) Melatonin is a potent modulator of dopamine release in the retina. Nature 306: 782–784

8 Finocchiaro L, Callebert J, Launay JM, Jallon JM (1988) Melatonin biosynthesis in *Drosophila*: its nature and its effect. J Neurochem 50: 382–387

9 Hamm HE, Meneaker M (1980) Retinal rhythms in chicks: Circadian variation in melatonin and serotonin N-acetyltransferase activity. Proc Natl Acad Sci USA 77: 4998–5002

10 Harlow HJ, Weekly BI (1986) Effect of melatonin on the force of spontaneous contractions of *in vitro* rat small and large intestine. J Pineal Res 3: 277–284

11 Hattori A, Wada M (1985) Diel changes in quail plasma concentration of melatonin under long day, short day and skeleton photoperiods. Proc of 10th Annual Meeting Japan Soc Comp Endocrinol pp 55

12 Huether G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. Experientia 49: 665–670

13 Kopp N, Claustrat B, Tappaz M (1980) Evidence for the presence of melatonin in the human brain. Neurosci Lett 19: 237–242

14 Leechin J (1988) Melatonin and other indoles in the rat pineal. J Chromatogr 428: 206–208

15 Legris GJ, Will PC, Hopfer U (1982) Inhibition of amiloride-sensitive sodium conductance by indoleamines. Proc Natl Acad Sci USA 79: 2046–2050

16 Pang SF, Yew DT (1979) Pigment aggregation by melatonin in the retinal pigment epithelium and choroid of guinea-pigs, *Cavia porcellus*. Experientia 35: 231–233

17 Pang SF, Chow PH, Wong TM, Tso ECF (1983) Diurnal variations of melatonin and N-acetylserotonin in the tissues of quails (*Coturnix* sp.), pigeons (*Columba livia*), and chickens (*Gallus domesticus*). Gen Comp Endocrinol 51: 1–7

18 Reiter RJ (1993) The melatonin rhythm: both a clock and a calendar. Experientia 49: 654–664

19 Reiter RJ, Richardson BA, Hurlbut EC (1981) Pineal, retinal and Harderian gland melatonin in a diurnal species, the Richardson's ground squirrel (*Spermophilus richardsonii*). Neurosci Lett 22: 285–288

20 Sallanon M, Claustrat B, Touret M (1982) Presence of melatonin in various cat brainstem nuclei determined by radioimmunoassay. Acta Endocrinol 101: 161–165

21 Vakkuri O, Rintamaki H, Leppaluoto J (1985) Presence of immunoreactive melatonin in different tissues of the pigeon (*Columba livia*). Gen Comp Endocrinol 58: 69–75

22 Vivien-Roels B, Pevet P (1993) Melatonin: presence and formation in invertebrates. Experientia 49: 642–647

23 Vivien-Roels B, Pevet P, Beck O, Fevre-Montange M (1984) Identification of melatonin in the compound eyes of an insect, the locust (*Locusta migratoria*), by radioimmunoassay and gas chromatography-mass spectrometry. Neurosci Lett 49: 153–157

24 Vivien-Roels B, Pevet P, Dubois MP, Arendt J, Brown GM (1981) Immunohistochemical evidence for the presence of melatonin in the pineal gland, the retina, and the Harderian gland. Cell Tissue Res 217:105–115.

25 Voisin P, Geffard M, Delaage M, Collin JP (1982) An immunological study of melatonin in the pineal organ, retina and plasma of the pigeon. Reprod Nutr Dev 22: 959–971

26 Zisapel N, Laudon M (1982) Dopamine release induced by electrical field stimulation of rat hypothalamus *in vitro*: inhibition by melatonin. Biochem Biophys Res Commun 104: 1610–1616